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Cardiorespiratory fitness and inflammatory profile on cardiometabolic risk in adolescents from the LabMed Physical Activity Study

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Abstract
Purpose: We investigated the combined effect of cardiorespiratory fitness and the clustered score of inflammatory biomarkers (InflaScore) on the cardiometabolic risk score in adolescents. Methods: This is a cross-sectional analysis with 529 adolescents (267 girls) aged 12–18 years. The shuttle run test was used to assess cardiorespiratory fitness. Continuous scores of clustered inflammatory biomarkers (high sensitivity C-reactive protein, complement factors C3 and C4, fibrinogen and leptin); cardiometabolic risk score (systolic blood pressure, triglycerides, ratio total cholesterol/HDL, HOMA-IR and waist circumference) were computed. Results: Adolescents with a higher inflammatory profile had the highest cardiometabolic risk score; adolescents with high InflaScore and low fitness had the highest odds of having a high cardiometabolic risk (OR 16.5; 95% CI 7.8–34.5), followed by adolescents with a higher InflaScore but fit (OR 7.5; 95% CI 3.7–8.4), and then by adolescents with a low InflaScore and unfit (OR 3.7; 95% CI 1.6–8.4) when compared to those with low InflaScore and fit, after adjustments for age, sex, pubertal stage, adherence to a Mediterranean dietary pattern and socioeconomic status. Conclusions: The findings of our study suggest that the combination of high inflammatory state and low cardiorespiratory fitness is synergistically associated with a significantly higher cardiometabolic risk score and thus supports the relevance of early targeted interventions to promote physical activity and preservation as part of primordial prevention.

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Cardiorespiratory Fitness and Inflammatory Profile on Cardiometabolic Risk in Adolescents from the LabMed Physical Activity Study.

Running Head

Cardiorespiratory Fitness, Inflammation and Cardiometabolic Risk

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The authors have no conflicts of interest relevant to this article to disclose. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Tables and Figures are at the end of the document

Abbreviations
ANCOVA: Analysis of covariance
BMI: body mass index
CVD: cardiovascular diseases
HOMA-IR: homeostatic model assessment of insulin resistance
Hs-CRP: High sensitivity C-reactive Protein
InflaScore: clustered score of inflammatory biomarkers
OR: odds ratio
WC: waist circumference

ABSTRACT

Purpose: We investigated the combined effect of cardiorespiratory fitness and the clustered score of inflammatory biomarkers (InflaScore) on the cardiometabolic risk score in adolescents.

Methods: This is a cross-sectional analysis with 529 adolescents (267 girls) aged 12-18 years. The shuttle run test was used to assess cardiorespiratory fitness. Continuous scores of clustered inflammatory biomarkers (High sensitivity C-reactive Protein, complements factors C3 and C4, fibrinogen and leptin); cardiometabolic risk score (systolic blood pressure, triglycerides, ratio total cholesterol/HDL, HOMA-IR and waist circumference) were computed.

Results: Adolescents with a higher inflammatory profile had the highest cardiometabolic risk score; adolescents with high InflaScore and low fitness had the highest odds of having a high cardiometabolic risk (OR=16.5; 95%CI: 7.8 to 34.5), followed adolescents with a higher InflaScore but fit (OR=7.5; 95%CI 3.7 to 8.4), then by adolescents with a low InflaScore and unfit (OR=3.7; 95%CI 1.6 to 8.4) when compared to those with low InflaScore and fit ,after adjustments for age, sex, pubertal stage, adherence to a Mediterranean dietary pattern and socioeconomic status.

Conclusions: The findings of our study suggest that the combination of high inflammatory state and low cardiorespiratory fitness is synergistically associated with a significantly higher cardiometabolic risk score and thus supports the relevance of early targeted interventions to promote physical activity and preservation as part of primordial prevention.

KEYWORDS: Inflammation, Cardiometabolic Risk, Aerobic fitness, Mediterranean diet, Youth.

Introduction

Atherosclerotic cardiovascular disease (CVD) is a multifactorial condition reflecting a lifelong pathological process.(Gersh, Sliwa, Mayosi, & Yusuf, 2010). Although the clinical manifestations of atherosclerosis occur mainly in adulthood, it is well-known that it has a long asymptomatic phase of development that begins early in life, often during childhood (Balagopal et al., 2011). Increased expression and activation of acute-phase proteins, complement factors (Barbu, Hamad, Lind, Ekdahl, & Nilsson, 2015), and adipocytokines have been associated with the development of atherosclerosis (Balagopal et al., 2011; Canas, Sweeten, & Balagopal, 2013). Chronic low-grade inflammation and activation of the immune system are involved in the pathogenesis of atherosclerosis (Balagopal et al., 2011), obesity-related insulin resistance, and type 2 diabetes (Esser, Legrand-Poels, Piette, Scheen, & Paquot, 2014). Indeed, increasing evidence shows that low-grade inflammation is associated with metabolic dysfunction, insulin resistance, and metabolic syndrome (Balagopal et al., 2011).

The increase in the prevalence and severity of metabolic syndrome in children and adolescents is likely to lower the age of onset and increase the incidence of CVD worldwide (Falahi, Hossein, Rad, & Roosta, 2015). Although different criteria have been proposed for the definition of metabolic syndrome in children and adolescents (Friend A, Craig L, 2013), the underlying concept of the various definitions is the same: a clustering of several CVD risk factors (e.g., waist circumference, hypertension, high levels of triglycerides, hyperglycemia, and high-density lipoprotein (HDL) cholesterol levels) (Andersen et al., 2015). In the absence of a universally accepted definition of metabolic syndrome in children and adolescents, the assessment of cardiometabolic risk in youth has been performed by summing standardized values (as a continuous variable) of individual CVD risk factors. This approach has been shown to be of value in the assessment of overall metabolic health in adolescents (Andersen et al., 2015; Bugge et al., 2012; Lobelo, Pate, Dowda, Liese, & Daniels, 2010).

In children and adolescents, cardiorespiratory fitness has been associated with positive metabolic outcomes (Ortega, Ruiz, Castillo, & Sjöström, 2008) and is considered an important determinant of current and future health status (Ortega et al., 2008; Ruiz et al., 2009). Some studies have investigated the association between cardiorespiratory fitness and inflammatory biomarkers and cardiometabolic risk factors separately in youth (Bugge et al., 2012; Martinez-Gomez et al., 2012; J Steene-Johannessen, Kolle, Andersen, & Anderssen, 2013; Jostein Steene-Johannessen, Anderssen, Kolle, & Andersen, 2009). For example, Martinez-Gomez and colleagues showed an inverse association between cardiorespiratory

fitness with C-reactive protein (hs-CRP) and complement factors C3 and C4, independent of age, sex, and body mass index (BMI) in European adolescents (Martinez-Gomez et al., 2012). In another study, Steene-Johannessen and colleagues showed a negative association between low cardiorespiratory fitness and CVD risk factors independent of pubertal stage (Jostein Steene-Johannessen et al., 2009). However, none of these studies considered the participants' dietary patterns in their analyses. In addition, these studies did not take into account the combined effect of low-grade inflammation and cardiorespiratory fitness on cardiometabolic health.

It is known that low-grade inflammation leads to several metabolic disorders, and cardiorespiratory fitness has been considered a powerful marker of metabolic health in children and adolescents (Ortega et al., 2008). Therefore, continuing this line of thought, it seems important to try to understand the interplay between cardiorespiratory fitness, low-grade inflammation, and cardiometabolic risk factors. Furthermore, we are not aware of any study that has evaluated the combined effect of a clustered inflammatory-biomarkers score and cardiorespiratory fitness on a cardiometabolic risk score independent of important potential confounders such as dietary pattern, pubertal status, and socioeconomic status.

Therefore, the present study aimed to investigate the combined effect of cardiorespiratory fitness and a clustered inflammatory-biomarkers score on a cardiometabolic risk score, controlling the analyses for potential confounders including adherence to a Mediterranean diet and socioeconomic status.

Methods

Study Design and Sample

The current report is part of the "Longitudinal Analysis of Biomarkers and Environmental Determinants of Physical activity (LabMed Physical Activity Study)", a school-based prospective cohort study carried out in four Portuguese cities from the North Region. Detailed description of sampling, recruitment approaches and data collection have been described elsewhere. (Agostinis-Sobrinho, Moreira, et al., 2016; Agostinis-Sobrinho, Santos, et al., 2016) In short, baseline data was collected in the fall of 2011, for 1,229 apparently healthy adolescents, i.e., participants without any medical diagnosis of physical or mental impairment, aged 12 to-18 years. Of the 1229 adolescents that agreed to participate in the LabMed study, 534 accepted to undergo blood collection. Of these, five individuals were excluded

due to hs-CRP values >10 mg/L, which may be indicative of acute inflammation or illness. Thus, leaving 529 adolescents (267 girls, 262 boys, mean age 14.3±1.7 years) as the final sample for the present report.

The LabMed Physical Activity Study was conducted in accordance with the Helsinki Declaration for Human Studies and approved by the Portuguese Data Protection Authority (#1112434/2011) and the Portuguese Ministry of Science and Education (0246200001/2011). All participants were informed of the study's goals, and written informed consent was obtained from participating adolescents and their parents or legal guardians.

Measures

Anthropometrics

Body height and weight were measured according to standard protocols (Lohman, Roche, Martorell, & Martorell, 1991)**,** with a portable stadiometer (Seca 213, Hamburg, Germany) and an electronic scale (Tanita Inner Scan BC 532, Tokyo, Japan), respectively. Body mass index (BMI) was calculated from the ratio of body weight (kg) to body height (m^2).

Waist Circumference (WC) measurements were taken midway between the lower rib margin and the anterior superior iliac spine at the end of normal expiration following standard procedures (Lohman et al., 1991).

Blood Pressure

Blood pressure was measured using a Dynamap vital signs monitors (model BP 8800, Critikon, Inc., Tampa, Florida). All adolescents were required to sit and rest for at least 5 min prior to the first blood pressure measurement. Participants were in a seated, relaxed position with their feet resting flat on the ground. Two measurements were taken, after five and 10 min of rest. The mean of these two measurements was considered. If the two measurements differed by 10 mmHg or more, a third measure was taken (McCrindle, 2010).

Pubertal stage

Participants self-assessed their pubertal stage of secondary sex characteristics (breast and pubic hair development for girls, genital and pubic hair development for boys; ranging from stage I to V), according to the criteria of Tanner and Whitehouse (Tanner & Whitehouse, 1976).

Socioeconomic Status

The socioeconomic status was assessed with the Family Affluence Scale (Currie et al., 2008).

Blood Sampling

Blood samples were obtained from each subject early in the morning, following a 10-hour overnight fast by venipuncture from the antecubital vein. The samples were stored in sterile blood collection tubes in refrigerated conditions (4° to 8°C) for no longer than 4 hours during the morning of collection and then sent to an analytical laboratory for testing according to standardized procedures, as follow: hs-C-Reactive Protein, latex enhanced immunoturbidimetric assay (Siemens Advia 1600/1800 Erlangen, Germany); HDL-Cholesterol, Precipitation of the Apolipoprotein B containing lipoproteins with dextran-magnesium-chloride (Siemens Advia 1600/1800 Erlangen, Germany); Glucose, Hexokinase method (Siemens Advia 1600/1800 Erlangen, Germany); Insulin, Chemiluminescence immunoassay (Siemens ACS Centaur System, Erlangen, Germany); Fibrinogen, Clauss method (Siemens BCS System, Erlangen, Germany); Complement factors C3 and C4, Immunoturbidimetric assay (Siemens Advia 1600/1800, Erlangen, German; Total cholesterol (TC) CHOD-POD enzymatic method (Siemens Advia 1600/1800); Triglycerides, enzyme glycerol phosphate oxidase method (GPO) (Siemens Advia 1600/1800 Erlangen, Germany). Leptin CRP, C3 and C4 were determined in serum and fibrinogen was determined in plasma. All assays were performed in duplicate according to the manufacturers' instructions.

Adherence to the Mediterranean Diet

To assess the degree of adherence to the Mediterranean diet, the KIDMED index (Mediterranean Diet Quality Index for children and adolescents) was used (Serra-Majem et al., 2004).

Cardiorespiratory Fitness

Cardiorespiratory fitness was assessed with the 20-metre Shuttle Run Test (20 m SRT) as described elsewhere (Leger, Mercier, Gadoury, & Lambert, 1988). We estimated the maximum oxygen consumption (VO2max, mL/kg/min) from the 20 m SRT number of laps performed using the Leger et al' equation (Leger et al., 1988).

Data management

The homeostatic model assessment (HOMA-IR), calculated as the product of basal glucose (mmol/L) and insulin (μlU/mL) levels divided by 22.5, was used as a proxy measure of insulin resistance. A continuous score representing a composite CVD risk factor profile was derived by summing the standardized values [Z-score = (participant's value - mean value of the sample) / standard deviation)] by age and sex, of triglycerides, systolic blood pressure, ratio total cholesterol/HDL-cholesterol, HOMA-IR and waist circumference as proposed for adolescents (Andersen et al., 2015; Bugge et al., 2012). High risk was considered when the individual had 1 SD ($\geq$ 3.1) above the mean of this composite CVD risk factor score as proposed by (Andersen et al., 2006).

We computed a continuous score of clustered inflammatory biomarkers (InflaScore) by summing the Z-scores by age and sex from the inflammatory biomarkers (C-Reactive Protein, C3, C4, fibrinogen and leptin) (Agostinis-Sobrinho, Moreira, et al., 2016). Participants were categorized into two groups: High InflaScore group (the first tertile) and Low InflaScore group (second and third tertiles).

The participants were divided into two cardiorespiratory fitness groups (Fit and Unfit) in according to proposed cut-off for this population (Ruiz et al., 2016). Then, according of the InflaScore groups (High and Low) and cardiorespiratory fitness group (Fit and Unfit), four exclusive groups were created (Low InflaScore/Fit, Low InflaScore/Unfit, High InflaScore/Fit and High InflaScore/Unfit).

Statistical Analysis

Descriptive data are presented as means and standard deviations. Independent Two-tailed *t*-Tests for continuous variables and Chi-square for categorical variables were used to examine the participant's differences according to inflammatory profile (High InflaScore and Low InflaScore) and cardiorespiratory fitness level (Fit and Unfit). Bivariate correlations were used as preliminary analysis to examine the associations between variables (see supplementary file, Table-S1).

Linear regression models were performed to determine the associations between the cardiometabolic risk score and (as the dependent variable) cardiorespiratory fitness or InflaScore (as predictor variables). We performed five linear regression models; Model 1 – unadjusted model; Model 2:

adjusted for age, sex, pubertal stage and socioeconomic status; Model 3: Model 2 additionally adjusted for adherence the Mediterranean diet; Model 4: Model 3 additionally cardiorespiratory fitness and InflaScore into the same model; Model 5- Model 4 additionally the interaction term of cardiorespiratory fitness and InflaScore. Standardized regression coefficients were used to express the β in the linear regression analyses

Analysis of covariance (ANCOVA) with Bonferroni post-hoc multiple comparison tests were used to assess the differences between mean values of cardiometabolic risk score across groups of InflaScore (High vs Low) stratified according to the cardiorespiratory fitness level (Fit and Unfit). Covariates included were age, sex, pubertal stage, adherence to a Mediterranean dietary pattern (KIDMED index) and socioeconomic status.

Binary logistic regression models were constructed to verify the relationship between high cardiometabolic risk score and the combined associations of cardiorespiratory fitness and a clustered score of inflammatory biomarkers. We performed three logistic regression models; Model 1: unadjusted model; Model 2: Model 1 additionally adjusted for age, sex, pubertal stage and socio-economic status; Model 3: Model 2 additionally adjusted adherence the Mediterranean diet.

Analysis was performed with the Statistical Package for the Social Sciences for Windows (Version 21.0 SPSS Inc., Chicago, IL). A p value < 0.05 denoted statistical significance.

Results

Descriptive characteristics of participants according to inflammatory profile and cardiorespiratory fitness level are presented in **Table 1**. Participants in the High InflaScore category had significantly higher BMI, WC, ratio total cholesterol/HDL, triglycerides, systolic blood pressure, leptin, cardiometabolic risk score and significantly lower HDL-Cholesterol and cardiorespiratory fitness (mL/kg/min) compared those who in low InflaScore category ($p<0.05$ for all). Unfit participants showed significantly lower levels of cardiorespiratory fitness and higher values for all the others cardiometabolic and inflammatory biomarkers and body composition variables compared with fit adolescents ($p<0.05$ for all).

……#INSERT table 1 here#.....

As depicted in Table 2, cardiorespiratory fitness was a negative predictor of cardiometabolic risk score (β=-0.254; p<0.001) and InflaScore was positively associated with cardiometabolic risk score (β=0.407; p<0.001) in the fully adjusted models (model 4). We also found a significant interaction between cardiorespiratory fitness and InflaScore (β=-0.535; p=0.024) in predicting cardiometabolic score (model 5).

……#INSERT table 2 here#.....

As shown in figure 1, unfit participants with high InflaScore had on average the highest cardiometabolic risk score ($F_{(3,519)}$ = 40,9 p<0,001) after adjustments for age, sex, pubertal stage, adherence to a Mediterranean dietary pattern and socioeconomic status. Fit participants with low InflaScore had on average the lowest cardiometabolic risk score.

….#INSERT Figure 1 here#.....

As shown in Table 3, adolescents classified as High InflaScore/Unfit had the highest odds of expressing high cardiometabolic risk score when compared to those of the Low InflaScore/Fit group (OR= 16.5; 95% CI: 7.8 - 34.5); also, participants classified as High InflaScore/Fit (OR=7.5; 95% CI 3.7 to 8.4), as well as those classified as Low InflaScore/Unfit (OR=3.7; 95% CI 1.6 to 8.4), were more likely to express a high cardiometabolic risk score, compared with those from Low InflaScore/Fit group after adjustments for age, sex, pubertal stage, socioeconomic status and adherence to a Mediterranean dietary pattern (model 3).

…#INSERT table 3 here#.....

Discussion

The main findings of the present study suggest that adolescents with a higher inflammatory profile had the highest cardiometabolic risk score; adolescents with a high InflaScore and low fitness had the highest odds of having high cardiometabolic risk factors after adjustments for age, sex, pubertal stage, adherence to a Mediterranean dietary pattern, and socioeconomic status.

Collectively, these findings suggest the existence of a close biological relationship between reduced clustered cardiometabolic risk factors and high cardiorespiratory fitness that is detectable as early as adolescence and that could be used to guide CVD prevention efforts and, consequently, the reduction of low-grade inflammation and/or protection against the low-grade inflammation process. The deleterious consequences ascribed to the low-grade inflammation process could be counteracted, to some extent, by maintaining appropriate levels of cardiorespiratory fitness.

Our regression analyses showed a significant interaction between cardiorespiratory fitness and InflaScore in predicting the cardiometabolic score, which means that the potential effects of InflaScore may indeed be moderated by cardiorespiratory fitness. Several studies have shown an inverse association between cardiorespiratory fitness and a clustering of CVD risk in adolescents (Enrique G. Artero et al., 2011; Lobelo et al., 2010; Moreira et al., 2013; Jostein Steene-Johannessen et al., 2009). However, we are not aware of prior studies examining the combined associations of cardiorespiratory fitness and a clustered inflammatory-biomarkers score on CVD risk factors in adolescents. In our logistic analysis, we showed that unfit participants with an adverse inflammatory profile were more likely to have (OR=16.5; $p < 0.05$ adjusted model) a high cardiometabolic risk score than those adolescents with an adverse inflammatory profile but who were fit (OR=7.5; $p<0.05$) compared to those with a low inflammatory profile who were fit. These findings provide new information for helping us to understand the role not only of weight status but also of cardiorespiratory fitness on cardiometabolic health in adolescents considering the inflammatory profile.

Recently, several studies have attempted to understand the independent impact of cardiorespiratory fitness on CVD risk scores in adolescents; this has been driven by analyses of the combined associations of cardiorespiratory fitness and weight status (BMI) on cardiometabolic risk (Enrique G. Artero et al., 2011; Lobelo et al., 2010). However, to clarify the context-dependence of cardiorespiratory fitness levels on CVD risk score, studies in different physiological states are needed.

Indeed, it's known that the inflammatory profile can be driven not only by adiposity but also by several other biological, behavioural, and environmental factors, such as lower birth weight (Labayen, Ortega, Sjöström, & Ruiz, 2009), poor diet (Schwingshackl & Hoffmann, 2014), stress, pollution, poor oral health, smoking, low socioeconomic status, and excessive alcohol consumption (Danesh et al., 2000; Engström, Hedblad, Janzon, & Lindgärde, 2007) among others. Continuing this line of thought, matching our analysis according to the inflammatory profile and cardiorespiratory fitness and instead considering only BMI provides a more comprehensive view of different physiological phenotypes. Additionally, BMI is a limited measure of obesity because it cannot distinguish between fat tissue and lean tissue.

Obesity, in particular excess visceral adiposity, is associated with insulin resistance, hyperglycaemia, dyslipidaemia, and hypertension, which together are referred to as metabolic syndrome (Esser et al., 2014). The metabolic syndrome has a multifactorial etiology, and the mechanisms underlying its onset are not completely understood (Friend A, Craig L, 2013; Rubin & Hackney, 2010). Our study demonstrated that the unfit group and the high InflaScore group both presented the poorest cardiometabolic profiles compared with respective groups (fit and low InflaScore). Recently, studies conducted with adolescents have reported that subjects with low cardiorespiratory fitness have higher CVD risks (Ruiz et al., 2009), higher odds of myocardial infarction late in life (Hogstrom, Nordstrom, & Nordstrom, 2014), and low fitness levels in adulthood (Ortega et al., 2013).

Our results were built upon previous studies showing that adolescents with high cardiorespiratory fitness levels and low inflammatory profile presented with the lowest cardiometabolic risk levels. These findings are particularly important from a public health perspective since atherosclerosis is considered an inflammatory disease (Bugge et al., 2012). The early identification of children and adolescents at risk for atherosclerosis may allow early intervention, thereby preventing or delaying the onset of CVD. In this sense, promoting high cardiorespiratory fitness levels may be an effective preventive strategy for the reduction of and protection against the low-grade systemic inflammation process.

Several studies have been conducted with normal weight or obese adolescents that aimed to test the association between cardiorespiratory fitness and single markers of inflammation, such as hs-CRP and complement factors C3, C4, and IL-6 (Andersen et al., 2010; Bugge et al., 2012; Martinez-Gomez et al., 2012; J Steene-Johannessen et al., 2013; Thomas & Williams, 2008). For example, Buchan and colleagues showed an inverse association between cardiorespiratory fitness; a clustered score of IL-6,

PAI-1, and CRP; adiponectin (inverted); and fibrinogen in 192 adolescents after adjustments for sex, age, physical activity, WC, and pubertal status (Buchan et al., 2015). However, of note is that these studies did not consider the confounding effect of adherence to a Mediterranean diet as we did.

In the last decade, nutritional and pharmacological strategies that inhibit the various inflammatory pathways responsible for obesity-associated metabolic complications and atherosclerosis in adulthood have been the subject of intensive research. High adherence to a Mediterranean diet has been associated with reduced all-cause and cardiovascular mortality risk (Gómez-gracia et al., 2013); and a recent review showed evidence that a strong adherence to a Mediterranean diet decreases inflammation and improves endothelial function (Schwingshackl & Hoffmann, 2014). The role of dietary intake on the prevention of cardiometabolic risk and inflammation in adolescents is also of importance since it is during the period of life that lifestyle habits are established. Important, however, is that our results remained significant even after the analysis was adjusted for adherence to a Mediterranean diet. One possible explanation why this variable had low affect in our models may be due to the fact that in our study 42.6% (data not showed) of our participants have an optimal adherence to a Mediterranean Diet. Indeed, it has been reported that adherence to the Mediterranean diet is prevalent in Portugal (Albuquerque et al., 2017).

As evidence has shown that precocious puberty is associated with a higher cardiometabolic risk (Widen et al., 2012), pubertal stage is an important confounding variable to take into consideration in epidemiological studies with adolescents. In addition, adolescence is a period where changes in physical activity, fitness, adiposity, and metabolic status, are likely to occur (Thomas & Williams, 2008). Indeed, pubertal stage was significantly associated with cardiometabolic risk score and its inclusion in our models increased the R^2, however, it did not change our main results (see supplementary file).

The strengths of our study include the novelty of the analysis of combined associations of cardiorespiratory fitness with a clustered inflammatory score on the cardiometabolic risk score of adolescents and the consideration of important confounding variables such as dietary patterns, socioeconomic status, and pubertal stage. The cardiorespiratory fitness tests used in our study were shown to be valid, reliable, and feasible for health-monitoring purposes in adolescents (Ruiz et al., 2009). In addition, we classified the participants in two cardiorespiratory fitness groups according to the sex-specific cut-off points recently proposed (Ruiz et al., 2016).

Limitations of this study include (i) its cross-sectional design, which precludes causal attributions; and (ii) the use of a single fasting baseline measurement of inflammatory biomarkers, as it is

possible that one measurement does not accurately reflect the state of chronic low-grade inflammation. Nevertheless, to minimize and improve this last point, we analysed not one but several biomarkers, which provided a more comprehensive assessment of the inflammatory state of the participants of our study. Apart from that, a composite continuum score of inflammatory biomarkers is becoming recognized and has already been proposed in children and adolescents (Agostinis-Sobrinho, Moreira, et al., 2016; E G Artero et al., 2013; Buchan et al., 2015). In addition, a cumulative marker of inflammation based on several markers might better assess true individual differences in overall inflammation than inflammatory markers studied individually (Arsenault et al., 2009).

In conclusion, results from this cross-sectional study suggest that the combination high inflammatory state and low cardiorespiratory fitness is synergistically associated with a significantly higher cardiometabolic risk score among Portuguese adolescents.

References

Agostinis-Sobrinho, C., Moreira, C., Abreu, S., Lopes, L., Sardinha, L. B., Oliveira-Santos, J., … Santos, R. (2016). Muscular fitness and metabolic and inflammatory biomarkers in adolescents: Results from LabMed Physical Activity Study. *Scandinavian Journal of Medicine & Science in Sports*, (October), 1–8. http://doi.org/10.1111/sms.12805

Agostinis-Sobrinho, C., Santos, R., Moreira, C., Abreu, S., Lopes, L., Oliveira-Santos, J., … Mota, J. (2016). Association between serum adiponectin levels and muscular fitness in Portuguese adolescents: LabMed Physical Activity Study. *Nutrition, Metabolism and Cardiovascular Diseases*, *6*(26), 517–524. http://doi.org/10.1016/j.numecd.2016.02.011

Albuquerque, G., Moreira, P., Rosário, R., Araújo, A., Teixeira, V. H., Lopes, O., … Padrão, P. (2017). Adherence to the Mediterranean diet in children: Is it associated with economic cost? *Porto Biomedical Journal*, *2*(4), 115–119. http://doi.org/10.1016/j.pbj.2017.01.009

Andersen, L. B., Harro, M., Sardinha, L. B., Froberg, K., Ekelund, U., Brage, S., & Anderssen, S. A. (2006). Physical activity and clustered cardiovascular risk in children: a cross-sectional study (The European Youth Heart Study). *Lancet (London, England)*, *368*(9532), 299–304. http://doi.org/10.1016/S0140-6736(06)69075-2

Andersen, L. B., Lauersen, J. B., Brønd, J. C., Anderssen, S. A., Sardinha, L. B., Steene-johannessen, J., … Ekelund, U. (2015). A New Approach to Define and Diagnose Cardiometabolic Disorder in Children, *2015*(539835).

Andersen, L. B., Müller, K., Eiberg, S., Froberg, K., Andersen, J. F. B., Bugge, A., … McMurray, R. G. (2010). Cytokines and clustered cardiovascular risk factors in children. *Metabolism*, *59*(4), 561–566.

Arsenault, B. J., Cartier, A., Côté, M., Lemieux, I., Tremblay, A., Bouchard, C., … Després, J.-P. (2009). Body Composition, Cardiorespiratory Fitness, and Low-Grade Inflammation in Middle-Aged Men and Women. *The American Journal of Cardiology*, *104*(2), 240–246. http://doi.org/10.1016/j.amjcard.2009.03.027

Artero, E. G., España-Romero, V., Jiménez-Pavón, D., Martinez-Gómez, D., Warnberg, J., Gómez-Martínez, S., … Molnar, D. (2013). Muscular fitness, fatness and inflammatory biomarkers in adolescents. *Pediatric Obesity*, *9*, 391–400.

Artero, E. G., Ruiz, J. R., Ortega, F. B., España-Romero, V., Vicente-Rodríguez, G., Molnar, D., … Gutiérrez, A. (2011). Muscular and cardiorespiratory fitness are independently associated with metabolic risk in adolescents: The HELENA study. *Pediatric Diabetes*, *12*, 704–712. http://doi.org/10.1111/j.1399-5448.2011.00769.x

Balagopal, P. B., De Ferranti, S. D., Cook, S., Daniels, S. R., Gidding, S. S., Hayman, L. L., … Steinberger, J. (2011). Nontraditional risk factors and biomarkers for cardiovascular disease: mechanistic, research, and clinical considerations for youth: a scientific statement from the American Heart Association. *Circulation*, *123*(23), 2749–2769. http://doi.org/10.1161/CIR.0b013e31821c7c64

Barbu, A., Hamad, O. A., Lind, L., Ekdahl, K. N., & Nilsson, B. (2015). The role of complement factor C3 in lipid metabolism. *Molecular Immunology*, *67*(1), 101–107. http://doi.org/10.1016/j.molimm.2015.02.027

Buchan, D. S., Boddy, L. M., Young, J. D., Cooper, S.-M., Noakes, T. D., Mahoney, C., … Baker, J. S. (2015). Relationships between Cardiorespiratory and Muscular Fitness with Cardiometabolic Risk in Adolescents. *Research in Sports Medicine*, *23*(3), 227–239. http://doi.org/10.1080/15438627.2015.1040914

Bugge, A., El-Naaman, B., McMurray, R. G., Froberg, K., Nielsen, C. H., Müller, K., & Andersen, L. B. (2012). Inflammatory markers and clustered cardiovascular disease risk factors in danish adolescents. *Hormone Research in Paediatrics*, *78*(5–6), 288–296.

Canas, J. A., Sweeten, S., & Balagopal, P. (2013). Biomarkers for cardiovascular risk in children. *Current Opinion in Cardiology*, *28*(2), 103–114. http://doi.org/10.1111/j.1365-2265.2012

Currie, C., Molcho, M., Boyce, W., Holstein, B., Torsheim, T., & Richter, M. (2008). Researching health inequalities in adolescents: The development of the Health Behaviour in School-Aged Children (HBSC) Family Affluence Scale. *Social Science and Medicine*, *66*, 1429–1436. http://doi.org/10.1016/j.socscimed.2007.11.024

Danesh, J., Whincup, P., Walker, M., Lennon, L., Thomson, A., Appleby, P., … Pepys, M. B. (2000). Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ (Clinical Research Ed.)*, *321*(7255), 199–204. http://doi.org/10.1136/bmj.321.7255.199

Engström, G., Hedblad, B., Janzon, L., & Lindgärde, F. (2007). Complement C3 and C4 in plasma and incidence of myocardial infarction and stroke: a population-based cohort study. *European Journal of Cardiovascular Prevention and Rehabilitation*, *14*(3), 392–397. http://doi.org/10.1097/01.hjr.0000244582.30421.b2

Esser, N., Legrand-Poels, S., Piette, J., Scheen, A. J., & Paquot, N. (2014). Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Research and Clinical Practice*, *105*(2), 141–150. http://doi.org/10.1016/j.diabres.2014.04.006

Falahi, E., Hossein, A., Rad, K., & Roosta, S. (2015). Diabetes & Metabolic Syndrome : Clinical Research & Reviews What is the best biomarker for metabolic syndrome diagnosis ? *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *9*(4), 366–372. http://doi.org/10.1016/j.dsx.2013.06.014

Friend A, Craig L, T. S. (2013). The Prevalence of Metabolic Syndrome in Children: A Systematic Review of the Literature. *Metab Syndr Relat Disord*, *11*(2), 71–80. http://doi.org/10.1089/met.2012.0122

Gersh, B. J., Sliwa, K., Mayosi, B. M., & Yusuf, S. (2010). Novel therapeutic concepts: The epidemic of cardiovascular disease in the developing world: Global implications. *European Heart Journal*, *31*(6), 642–648. http://doi.org/10.1093/eurheartj/ehq030

Gómez-gracia, E., Ph, D., Ruiz-gutiérrez, V., Ph, D., Fiol, M., & Ph, D. (2013). Primary Prevention of Cardiovascular Disease with a Mediterranean Diet. *The New England Journal of Medicine*, *368*(14), 1279–1290. http://doi.org/10.1056/NEJMoa1200303

Hogstrom, G., Nordstrom, A., & Nordstrom, P. (2014). High aerobic fitness in late adolescence is associated with a reduced risk of myocardial infarction later in life: a nationwide cohort study in men. *Eur.Heart J.*, *35*(44), 3133–3140. http://doi.org/10.1093/eurheartj/eht527

Labayen, I., Ortega, F. B., Sjöström, M., & Ruiz, J. R. (2009). Early life origins of low-grade inflammation and atherosclerosis risk in children and adolescents. *The Journal Of Pediatrics*, *155*(5), 673–677. http://doi.org/10.1016/j.jpeds.2009.04.056

Leger, L. A., Mercier, D., Gadoury, C., & Lambert, J. (1988). The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci*, *6*(2), 93–101. http://doi.org/10.1080/02640418808729800

Lobelo, F., Pate, R. R., Dowda, M., Liese, A. D., & Daniels, S. R. (2010). Cardiorespiratory fitness and clustered cardiovascular disease risk in U.S. adolescents. *Journal of Adolescent Health*, *47*(4), 352–359. http://doi.org/10.1016/j.jadohealth.2010.04.012

Lohman, T. G., Roche, A. F., Martorell, F., & Martorell, R. (1991). *Anthropometric standardization reference manual*. *Human Kinetics Book:Champaign, IL,*. Champaign IL: Human Kinetics Book.

Martinez-Gomez, D., Gomez-Martinez, S., Ruiz, J. R., Diaz, L. E., Ortega, F. B., Widhalm, K., … Marcos, A. (2012). Objectively-measured and self-reported physical activity and fitness in relation to inflammatory markers in European adolescents: The HELENA Study. *Atherosclerosis*, *221*(1), 260–267. http://doi.org/10.1016/j.atherosclerosis.2011.12.032

McCrindle, B. W. (2010). Assessment and management of hypertension in children and adolescents. *Nature Reviews Cardiology*, *7*(3), 155–163. http://doi.org/10.1038/nrcardio.2009.231

Moreira, C., Santos, R., Moreira, P., Lobelo, F., Ruiz, J. R., Vale, S., … Mota, J. (2013). Cardiorespiratory fitness is negatively associated with metabolic risk factors independently of the adherence to a healthydietary pattern. *Nutrition, Metabolism and Cardiovascular Diseases*, *23*(7), 670–676. http://doi.org/10.1016/j.numecd.2012.01.011

Ortega, F. B., Ruiz, J. R., Castillo, M. J., & Sjöström, M. (2008). Physical fitness in childhood and adolescence: a powerful marker of health. *International Journal of Obesity (2005)*, *32*(1), 1–11. http://doi.org/10.1038/sj.ijo.0803774

Ortega, F. B., Ruiz, J. R., Labayen, I., Hurtig-Wennlöf, A., Harro, J., Kwak, L., … Sjöström, M. (2013). Role of socio-cultural factors on changes in fitness and adiposity in youth: A 6-year follow-up study. *Nutrition, Metabolism and Cardiovascular Diseases*, *23*(9), 883–890. http://doi.org/10.1016/j.numecd.2012.05.008

Rubin, D. A., & Hackney, A. C. (2010). Inflammatory cytokines and metabolic risk factors during growth and maturation: Influence of physical activity. *Med Sport Sci*, *55*, 43–55. http://doi.org/10.1159/000321971

Ruiz, J. R., Castro-Piñero, J., Artero, E. G., Ortega, F. B., Sjöström, M., Suni, J., & Castillo, M. J. (2009). Predictive validity of health-related fitness in youth: a systematic review. *British Journal of Sports Medicine*, *43*(12), 909–923. http://doi.org/10.1136/bjsm.2008.056499

Ruiz, J. R., Cavero-Redondo, I., Ortega, F. B., Welk, G. J., Andersen, L. B., & Martinez-Vizcaino, V. (2016). Cardiorespiratory fitness cut points to avoid cardiovascular disease risk in children and adolescents; what level of fitness should raise a red flag? A systematic review and meta-analysis. *British Journal of Sports Medicine*, *50*, 1451–1458. http://doi.org/10.1136/bjsports-2015-095903

Schwingshackl, L., & Hoffmann, G. (2014). Mediterranean dietary pattern, inflammation and endothelial function: A systematic review and meta-analysis of intervention trials. *Nutrition, Metabolism and Cardiovascular Diseases*, *24*(9), 929–939. http://doi.org/10.1016/j.numecd.2014.03.003

Serra-Majem, L., Ribas, L., Ngo, J., Ortega, R. M., García, A., Pérez-Rodrigo, C., & Aranceta, J. (2004). Food, youth and the Mediterranean diet in Spain. Development of KIDMED, Mediterranean Diet Quality Index in children and adolescents. *Public Health Nutrition*, *7*, 931–935. http://doi.org/10.1079/PHN2004556

Steene-Johannessen, J., Anderssen, S. A., Kolle, E., & Andersen, L. B. (2009). Low muscle fitness is associated with metabolic risk in youth. *Med Sci Sports Exerc*, *41*(7), 1361–1367. http://doi.org/10.1249/MSS.0b013e31819aaae5

Steene-Johannessen, J., Kolle, E., Andersen, L. B., & Anderssen, S. A. (2013). Adiposity, aerobic fitness, muscle fitness, and markers of inflammation in children. *Med Sci Sports Exerc*, *45*(4), 714–721. http://doi.org/10.1249/MSS.0b013e318279707a

Tanner, J. M., & Whitehouse, R. H. (1976). Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child*, *51*(3), 170–179. http://doi.org/10.1136/adc.51.3.170

Thomas, N. E., & Williams, D. R. R. (2008). Inflammatory factors, physical activity, and physical fitness in young people: Review. *Scandinavian Journal of Medicine and Science in Sports*, *18*(5), 543–556. http://doi.org/10.1111/j.1600-0838.2008.00824.x

Widen, E., Silventoinen, K., Sovio, U., Ripatti, S., Cousminer, D. L., Hartikainen, A.-L. L., … Palotie, A. (2012). Pubertal timing and growth influences cardiometabolic risk factors in adult males and females. *Diabetes Care*, *35*(4), 850–856. http://doi.org/10.2337/dc11-1365

Figure 1: Cardiometabolic risk factor across Inflammatory clustered score profile groups (Low InflaScore and High InflaScore) by Cardiorespiratory fitness groups (Fit vs Unfit). Bars represent adjusted means and 95% confidence intervals, for age, sex, pubertal stage, adherence to the Mediterranean diet and socio-economic status, as confounders.

Figure 1 here#

a Significantly different from all the others groups ($p<0.01$).

b Significantly different from the High InflaScore and Unfit group and Low InflaScore and Fit group ($p<0.01$).

Table 1. Participants' characteristics in according to the inflammatory profile and cardiorespiratory fitness status

Characteristics	Low InflaScore (n=352)	High InflaScore (n=177)	Unfit (n=135)	Fit (n=394)
Age (year)	14.30(±1.72)	14.39(±1.75)	15.0(±1.7)	14.3(±1.6)
Weight (Kg)	52.41(±11.06) [a]	60.6(±14.3)	61.5(±13.9) [b]	52.9(±11.6)
Height (cm)	160.2(±9.6)	160.4(±9.6)	160.1(±8.9)	160.0(±9.8)
BMI (kg m^2)	20.27(±3.0) [a]	23.4(±4.41)	23.6(±4.3) [b]	20.5(±3.3)
Waist circumference	70.2(±7.6) [a]	78.8(±12.1)	78.6 (±12.2) [b]	71.2 (±8.7)
Pubertal status A: ≤III/ IV/ V (%)	38.1/48.9/13.1	46.2/ 40.2/ 13.6	38.5/ 42.2/ 19.3	40.6/ 48.2/11.2
Pubertal status B: ≤III/ IV/ V (%)	28.7/ 49.5/ 21.8	28/52.3/19.7	23.7/ 50.4/ 25.9	30.5/49.2/20.3
C3 (mg/dL)	110.25(11.4) [a]	131.8 (± 13.8)	122.5(± 15.96) [b]	115.6(±15.7)
C4 (mg/dL)	18.4(±4.6) [a]	25.4 (± 6.3)	22.6 (± 6.4) [b]	20.1 (± 5.9)
CRP mg/L	0.30(±0,4) [a]	2.25 (±2.7)	1.41(±2.1) [b]	0.78(±1.73)
Fibrinogen (mg/dL)	244 (± 28.2) [a]	302.5 (± 41.8)	277.0 (± 42.2) [b]	259.7(±42.7)
HDL-Cholesterol (mg/dL)	55.6(±11.8) [a]	51.6(±11.7)	53,6(±11.9)	54,6(±11.9)
Insulin resistance (HOMA-IR)	3.30(±6.4)	3.74(±2.0)	4.56(±10.23) [b]	3.07(±1.57)
Leptin (ng/mL)	2.82 (± 2.9) [a]	6.7 (± 6.8)	6.6 (± 6.6) [b]	3.2 (± 4.0)
Ratio Total cholesterol/HDL	2.80(±0.5) [a]	3.05(±0.6)	2.96(±0.6) [b]	2.86(±0.5)
Triglycerides (mg/dL)	64.3(±30.17) [a]	74.5(±35.3)	68.9(±37.3)	67.2(±30.4)
Systolic Blood Pressure (mm Hg)	117.8(±12.3) [a]	122.0(±12.8)	121.2(±13.2) [b]	118.5(±12.4)
Cardiometabolic risk score	-0.85(±-2.43) [a]	1.60 (±3.5)	1.24(±3.6) [b]	-0.47(±2.7)
Cardiorespiratory fitness – VO_2max (mL/kg/min)	43.1(±6.8) [a]	40.1(±6.4)	35.1(±3.6) [b]	44.45(±5.9)
Cardiorespiratory fitness (#laps – 20mSRT)	48.9(±25.6) [a]	38.4(±23.5)	25.4(±11.2) [b]	51.6(±25.3)
Socioeconomic Status	6.5(±1.7)	6.2(±1.6)	6.0(±1.7)	6.5(±1.8)
KIDMED Index	7.12(±2.05)	7.07(±1.96)	6.6(±1.7)	7.2(±1.8)

Data are mean and standard deviations.

[a]Significantly different from High InflaScore ($p<0.05$), [b]Significantly different from High CRF ($p<0.05$) - Independent Two-tailed *t*-Tests for continuous variable and chi-square for categorical variables.

BMI: body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; CRP, C - reactive protein; SRT: shuttle run test; KIDMED Index, adherence to the Mediterranean index.

Pubertal stage- A - breast development in girls; genital development in boys. Pubertal stage-B - pubic hair development

Table 2. Regression coefficients examining the association of Clustered Score of Inflammatory Biomarkers and Cardiorespiratory Fitness on Cardiometabolic risk score.

	Cardiometabolic risk score (dependent variable)		
	β	*P*-value	R^2
Model 1			
Cardiorespiratory fitness	-0.274	<0.001	0.075
InflaScore	0.461	<0.001	0.211
Model 2			
Cardiorespiratory fitness	-0.385	<0.001	0.110
InflaScore	0.470	<0.001	0.225
Model 3			
Cardiorespiratory fitness	-0.396	<0.001	0.120
InflaScore	0.469	<0.001	0.230
Model 4			
Cardiorespiratory fitness	-0.254	<0.001	0.265
InflaScore	0.407	<0.001	
Model 5			
Cardiorespiratory fitness x InflaScore	-0.534	0.024	0.271

β: Standardized regression coefficients
Model 1- Unadjusted model
Model 2- Adjusted for age, sex, pubertal stage and socioeconomic status.
Model 3- Model 2 additionally adherence to the Mediterranean diet.
Model 4- Model 3 additionally cardiorespiratory fitness and InflaScore into the same model.
Model 5- Model 4 additionally the interaction term of cardiorespiratory fitness and InflaScore

Table 3. Odds ratio of high cardiometabolic risk score by cardiorespiratory fitness and inflammatory profile.

Parameter		High cardiometabolic risk score (n=77)					
		Model 1	*P*-value	Model 2	*P*-value	Model 3	P-value
Low InflaScore	Fit (n=286)	1		1		1	
	Unfit (n=66)	3.1(1.4 - 6.7)	<0.001	3.70 (1.6 - 8.3)	<0.001	3.7 (1.6 - 8.4)	0.002
High InflaScore	Fit (n=108)	6.6 (3.3 - 13.3)	<0.001	7.5 (3.7 - 15.4)	<0.001	7.5 (3.7 - 8.4)	<0.001
	Unfit (n=69)	13.6 (6.7 - 27.5)	<0.001	16.4 (7.8 - 34.4)	<0.001	16.5 (7.8 - 34.5)	<0.001

OR, odds ratios; CI, confidence intervals; 1, reference category.

Model 1- Unadjusted model

Model 2- Adjusted for age, sex, pubertal stage and socioeconomic status.

Model 3- model 1 additionally adherence to the Mediterranean diet

Table S 1. Bivariate correlations between cardiometabolic variables, pubertal stage, kidmed index and socioeconomic status

	InflaScore	Cardiometabolic risk score	Pubertal status A	Pubertal status B	Kidmed index	Socioeconomic status
Cardiorespiratory fitness	**-0.248****	**-0.274***	**-0.123****	**-0.174***	**0.092***	0.067
InflaScore[a]	-	**0.461****	-0.026	-0.031	0.013	-0.051
Cardiometabolic risk score[b]		-	**0.095***	**0.090***	0.025	0.042

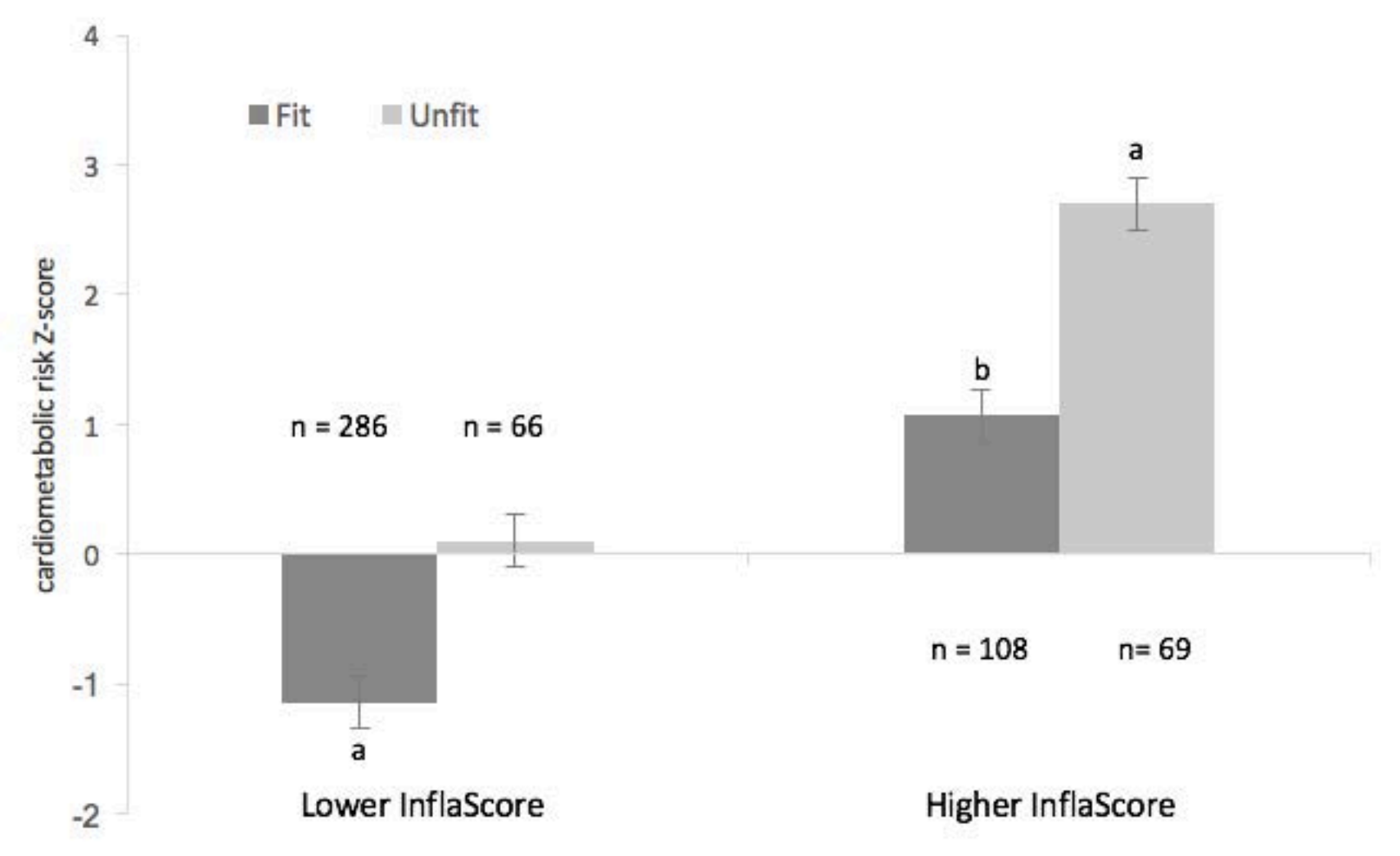
KIDMED index, adherence to the Mediterranean diet; Cardiorespiratory fitness, VO2max, mL/kg/min; Pubertal stage- A - breast development in girls; genital development in boys. Pubertal stage-B - pubic hair development

[a]Clustered Score of Inflammatory Biomarkers was computed as the sum of the following z-scores by age and sex: C - reactive protein + Complement factor C3 + Complement factor C4 + Leptin + Fibrinogen.

[b]Clustered score of cardiometabolic risk factors was computed as the sum of the following z-scores by age and sex: triglycerides + systolic blood pressure + ratio total cholesterol/HDL-cholesterol + HOMA-IR + waist circumference.

** $p < 0.01$.

* $p < 0.05$.


4
3
2
1
0
-1
-2
cardiometabolic risk Z-score
Fit
Unfit
a
b
a
n = 286
n = 66
n = 108
n= 69
Lower InflaScore
Higher InflaScore